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Novel genetic markers improve measures of atrial fibrillation risk prediction

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Aims Atrial fibrillation (AF) is associated with adverse outcome. Whether recently discovered genetic risk markers improve AF risk prediction is unknown.

Methods and results We derived and validated a novel AF risk prediction model from 32 possible predictors in the Women's Health Study (WHS), a cohort of 20 822 women without cardiovascular disease (CVD) at baseline followed prospectively for incident AF (median: 14.5 years). We then created a genetic risk score (GRS) comprised of 12 risk alleles in nine loci and assessed model performance in the validation cohort with and without the GRS. The newly derived WHS AF risk algorithm included terms for age, weight, height, systolic blood pressure, alcohol use, and smoking (current and past). In the validation cohort, this model was well calibrated with good discrimination [C-index (95% CI) = 0.718 (0.684–0.753)] and improved all reclassification indices when compared with age alone. The addition of the genetic score to the WHS AF risk algorithm model improved the C-index [0.741 (0.709–0.774); $P = 0.001$], the category-less net reclassification [0.490 (0.301–0.670); $P < 0.0001$], and the integrated discrimination improvement [0.00526 (0.0033–0.0076); $P < 0.0001$]. However, there was no improvement in net reclassification into 10-year risk categories of <1, 1–5, and 5+% [0.041 (−0.044–0.12); $P = 0.33$].

Conclusion Among women without CVD, a simple risk prediction model utilizing readily available risk markers identified women at higher risk for AF. The addition of genetic information resulted in modest improvements in predictive accuracy that did not translate into improved reclassification into discrete AF risk categories.

Keywords Women • Atrial fibrillation • Genetics • Risk prediction • Epidemiology

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and is associated with enormous societal costs, including an increased risk of stroke, heart failure (HF), and death.[1–3] The prevalence of AF is increasing even among those individuals thought to be at low risk, such as women or those without cardiovascular disease (CVD) or HF.[4,5] Treatment of AF remains challenging and associated with risk; therefore, prevention is an important public health objective. Recently, investigators working in the Framingham Heart Study (FHS) and the Atherosclerosis Risk in Communities (ARIC) study derived separate AF risk prediction models among individuals with and without heart disease, but neither study considered routine blood biomarkers or genetic markers for inclusion in those models.[6–11] These prediction algorithms also require electrocardiograms (ECGs), which may not be readily available among individuals without clinical heart disease.

The first aim of this study was to derive and validate an AF risk prediction algorithm that could be employed in our healthy population of 20 822 women without prevalent CVD, HF, or ECGs at baseline. The second aim of the study was to determine whether a genetic risk score (GRS) based on recently published risk

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alleles[11] could improve AF risk prediction beyond traditional risk factors and biomarkers among this population without established CVD, where genetic factors might contribute a greater proportion of risk.

Methods

Study participants

Study participants were American female health professionals enrolled in the Women's Genome Health Study (WGHS), a subset of the Women's Health Study (WHS), and included 20 822 women of European ancestry for whom genetic information was available and who did not have CVD, HF, or AF at baseline. Details of the design of the WHS and the WGHS are contained in the Supplementary material online. All participants provided written informed consent, and the study complies with the Declaration of Helsinki and was approved by the institutional review board of the Brigham and Women's Hospital.

Endpoint ascertainment

The methods of AF ascertainment have been reported previously[3] and are described in detail in the Supplementary material online. Briefly, women were asked to report date of any AF diagnosis at enrolment, at 48 months, and then annually thereafter. Those reporting an AF event were asked for permission to obtain medical records, which were then reviewed by a physician endpoint committee to confirm AF. Only confirmed events are included in the present analysis.

Laboratory evaluation and genotyping

Assay characteristics for plasma biomarkers and details of the genotyping and imputation methods are contained in the Supplementary material online.

Derivation and validation of a novel atrial fibrillation prediction algorithm

Of the 20 822 WGHS participants eligible for this study, two-thirds ($n = 13\,743$) were randomly assigned to the model derivation data set, and the remaining one-third ($n = 7079$) were reserved as an independent validation data set. Variables considered for inclusion in the AF risk prediction algorithm are displayed in *Table 1* and include traditional and lifestyle risk factors easily measured in clinical practice as well as available biomarkers. In the model derivation set, participants without complete information on these variables were excluded ($n = 682$), for a total sample size of 13 061, including 404 validated cases of AF. The best model was fit using Cox proportional hazards models with both forward and backward stepwise procedures for variable selection. Minimization of the Bayes Information Criteria (BIC)[12] was utilized to select covariates for inclusion. Because the BIC imposes a penalty for each additional covariate added to a model, the number of covariates included was also limited. The final WHS AF risk prediction model was then tested for discrimination (Harrell c-index)[13] and calibration (Nam and D'Agostino modification of the Hosmer–Lemeshow goodness-of-fit statistic)[14] in the validation set. Participants without complete information on the covariates selected for inclusion in the final WHS model were excluded ($n = 200$) for a total sample size in the validation set of 6879, including 212 cases of AF. In exploratory secondary analyses, the GRSs described below were added to the list of variables considered for inclusion in the derivation cohort.

Genetic risk score

Twelve single-nucleotide polymorphisms (SNPs) in nine loci reported to associate with AF were included in the GRS.[11,15,16] Seven of the SNPs (rs13376333, rs2200733, rs10033464, rs3853445, rs3807989, rs7164883, and rs7193343) were directly genotyped, while the rest (rs3903239, rs17570669, rs10821415, rs10824026, and rs1152591) were imputed. In the primary analysis, a weighted GRS was created by summing the product of the natural logarithm of the published risk ratio for each SNP (Supplementary material online, *Table S1*) times the gene dose at that SNP for each participant. Because allele weights were calculated by taking the natural logarithm of published risk ratios, alleles with risk ratios >1 had positive weights, while those with risk ratios <1 had negative weights. In order to eliminate bias, risk estimates from replication (rather than discovery) cohorts were used wherever possible. As a secondary analysis, an unweighted GRS was constructed to evaluate the sensitivity of our results to these published risk estimates. For this score, the allele associated with increased AF risk at each SNP was identified, and the measured or imputed allele dose at each of the 12 SNPs was then summed for each participant.

Clinical reclassification of atrial fibrillation prediction models

The models developed in the derivation set were used to estimate the 10-year risk of AF in the validation set ($n = 6879$) and improvement in measures of discrimination and calibration with the addition of clinical and/or genetic covariates were calculated in this cohort. While there is no broad consensus on what risk categories are clinically informative, 10-year clinical risk categories of <1, 1 to <5, and 5% and higher were utilized on an *a priori* basis given the low-risk nature of this healthy population.[6,17,18] To test whether the WHS score and/or the addition of genetic information improved clinical risk classification across categories, the net reclassification improvement (NRI) and the reclassification calibration test were calculated.[19] To address potential finer increments in reclassification, the continuous NRI and the integrated discrimination improvement (IDI) were calculated for each base model with and without genetic information.[18] Modifications appropriate for survival data were used.[18,20] Bootstrap resampling was used to calculate confidence intervals and *P*-values for each discrimination and reclassification statistic. As a sensitivity analysis, changes in WHS AF risk prediction algorithm performance after the addition of genetic information were calculated in all available women in the WGHS cohort with complete information on all model covariates ($n = 20\,222$). Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Study characteristics

The derivation and validation cohorts were similar with respect to the baseline characteristics and variables considered for inclusion in the AF risk prediction algorithm (*Table 1*). Established AF risk factors such as age, body mass index, weight, height, hypertension, and alcohol use were similar in the two cohorts. Levels of biomarkers previously associated with AF such as markers of inflammation, haemoglobin A1c, creatinine, and lipids were similar in the two cohorts.

Table 1 Baseline characteristics and covariables considered for inclusion in the atrial fibrillation risk prediction algorithm among women in the derivation and validation cohorts

Characteristic	Derivation cohort (n = 13 061)	Validation cohort (n = 6879)
Events/person-years of follow-up	404/181 350	212/95 253
Incidence rate, per 1000 person-years of observation	2.23	2.23
Age [median (IQR), years]	52.9 (48.9–58.8)	52.9 (48.9–58.8)
Body mass index [median (IQR), kg/m^2]	24.8 (22.5–28.3)	24.9 (22.5–28.3)
Weight [median (IQR), kg]	67.1 (59.9–77.1)	68.0 (59.9–77.1)
Height [median (IQR), cm]	165.0 (159.9–167.5)	165.0 (159.9–167.5)
Systolic blood pressure [median (IQR), mmHg]	125 (115–135)	125 (115–135)
Diastolic blood pressure [median (IQR), mmHg]	80 (70–80)	80 (70–80)
Physical activity [*n* (%)]		
1–3 times per week	4193 (32.1)	2263 (32.9)
4+ times per week	1520 (11.6)	798 (11.5)
Ever smoker [*n* (%)]	6375 (48.8)	3338 (48.5)
Alcohol use, 2+ drinks/day [*n* (%)]	527 (4.0)	289 (4.2)
History of hypertension [*n* (%)]	3143 (24.1)	1640 (23.9)
History of treatment for high blood pressure [*n* (%)]	1618 (12.4)	874 (12.7)
History of treatment for high cholesterol [*n* (%)]	387 (3.0)	246 (3.6)
History of diabetes [*n* (%)]	314 (2.4)	163 (2.4)
History of menopause [*n* (%)]	7005 (53.6)	3729 (54.3)
Hormone therapy use [*n* (%)]	5827 (44.6)	3008 (43.8)
Aspirin use [*n* (%)]	6553 (50.2)	3458 (50.3)
Vitamin E use [*n* (%)]	6502 (49.8)	3500 (50.9)
Beta carotene use [*n* (%)]	6535 (50.1)	3450 (50.2)
Cholesterol [median (IQR), mmol/L]		
Total	5.39 (4.74–6.11)	5.39 (4.77–6.09)
Low-density lipoprotein	3.13 (2.59–3.72)	3.14 (2.61–3.74)
High-density lipoprotein	1.35 (1.13–1.62)	1.35 (1.12–1.62)
Non-high-density lipoprotein	3.98 (3.33–4.69)	3.99 (3.34–4.71)
Triglycerides [median (IQR), mmol/L]	1.33 (0.94–1.95)	1.33 (0.95–1.97)
Apolipoprotein B100 [median (IQR), g/L]	0.995 (0.834–1.208)	1.001 (0.841–1.207)
Apolipoprotein A-I [median (IQR), g/L]	1.494 (1.329–1.683)	1.498 (1.325–1.684)
Lipoprotein(a) [median (IQR), μmol/L]	0.37 (0.15–1.13)	0.37 (0.15–1.15)
hsCRP [median (IQR), mg/L]	2.0 (0.8–4.4)	2.0 (0.8–4.2)
s-ICAM-1 [median (IQR), μg/L]	341.2 (300.2–393.0)	343.2 (302.8–394.5)
Fibrinogen [median (IQR), μmol/L]	10.29 (9.02–11.77)	10.21 (8.97–11.75)
Homocysteine [median (IQR), μmol/L]	10.4 (8.7–12.9)	10.5 (8.7–12.8)
Creatinine [median (IQR), μmol/L]	54.1 (48.0–61.0)	53.9 (48.2–60.7)
Haemoglobin A1c [median (IQR), %]	4.99 (4.83–5.17)	4.99 (4.83–5.17)

Women's Health Study atrial fibrillation model derivation and validation

In the derivation cohort, 32 potential variables outlined in *Table 1* were evaluated for model inclusion. Univariable association between each potential variable and incident AF in the derivation cohort are presented in Supplementary material online, *Table S2*. Of these, the inclusion of terms for the natural logarithm of age, weight, height, systolic blood pressure, ≥2 alcoholic drinks per day, and a history of either current or past smoking (ever smokers) resulted in the best fitting prediction model with the smallest BIC (7319.7). Model coefficients from the derivation cohort for these variables are presented in *Table 2*. The BIC for a model including the body mass index instead of height and weight was 7347.8, and the BIC for a model including age instead of the natural logarithm of age was 7321.8. Although none of the blood-based biomarkers were included in the final model, high-sensitivity C-reactive protein (hsCRP) would have been the next variable included ($P = 0.02$), but inclusion resulted in a small increase in the BIC (BIC = 7320.7 with hsCRP).

We then tested this AF prediction model in the validation cohort. Using coefficients calculated in the derivation set, the c-index (95% CI) for the WHS predictive model [0.718 (0.684–

Table 2 Beta-coefficients and multivariable adjusted hazard ratios for atrial fibrillation for each covariate selected for inclusion in the WHS atrial fibrillation risk prediction model

Base model covariables	Beta (SE)	Adjusted HR (95% CI)	*P*-value
Age	0.0924 (0.0060)	1.10 (1.08–1.11)	<0.0001
WHS model			
Ln(age)[a]	5.480 (0.40)	239.79 (109.96–522.94)	<0.0001
Weight (per 10 kg)	0.157 (0.035)	1.17 (1.09–1.25)	<0.0001
Height (per 10 cm)	0.306 (0.082)	1.36 (1.16–1.60)	0.0002
Systolic blood pressure, (per 10 mmHg)	0.155 (0.037)	1.17 (1.09–1.26)	<0.0001
2+ drinks per day	0.491 (0.20)	1.63 (1.10–2.43)	0.015
Ever smoker	0.254 (0.10)	1.29 (1.06–1.57)	0.01

Coefficients displayed here were calculated in the derivation cohort and were used to test the model in the validation cohort.
[a]For context, a 10-year increase in age (e.g. from age 50 to 60) would be associated with a 2.72-fold increase in atrial fibrillation risk.

Table 3 Fit, calibration, and discrimination statistics for the age and WHS atrial fibrillation risk prediction models in the validation cohort

	Risk prediction algorithm		
	Age alone	WHS	*P*-value
Model fit (χ^2)[a]	55.3	87.9	—
Model calibration [χ^2(*P*-value)][b]	7.01 (0.54)	8.07 (0.43)	—
C-index (95% CI)	0.671 (0.636–0.710)	0.718 (0.684–0.753)	<0.0001
NRI (95% CI)		0.211 (0.117–0.303)	<0.0001
Continuous NRI (95% CI)		0.578 (0.406–0.751)	<0.0001
IDI		0.0064 (0.0045–0.0088)	<0.0001

CI, confidence interval; IDI, integrated discrimination improvement; NRI, net reclassification improvement; WHS, Women's Health Study.
[a]Each likelihood ratio χ^2 statistic was highly significant ($P < 0.0001$).
[b]*P*-value of <0.01 required to reject the hypothesis that a model is well calibrated.

0.753)] was significantly better than that for age alone [0.671 (0.636–0.710), $P < 0.0001$], and the model was well calibrated ($P = 0.43$). The WHS AF risk score substantially improved classification into 10-year risk categories of <1, 1 to <5, and 5+%, with an NRI of 0.211 ($P < 0.0001$) and resulted in a significant improvement in the continuous NRI and IDI (*Table 3*, *Figure 1*).

Genetic risk scores and atrial fibrillation risk

The association with incident AF across quintiles of the weighted and unweighted GRS in the entire WHS ($n = 20\,347$) is displayed in *Figure 2*. As shown, women in the top quintile of the weighted and unweighted score had a 2.25-fold (95% CI: 1.75–2.90, *P*-trend < 0.0001) and a 2.85-fold (95% CI: 2.18–3.73, *P*-trend < 0.0001) increase in the risk of AF, respectively, after adjustment for the WHS prediction model covariates. The per-allele relative risks for each of the individual SNPs included in the GRS are displayed in Supplementary material online, *Table S3*. When modelled as continuous variables, both the weighted and the unweighted GRS were significantly associated with incident AF in the validation and entire cohort (each $P < 0.0001$). When either the weighted or unweighted score was included among the candidate AF risk predictors considered for inclusion in a secondary, exploratory AF risk prediction model, each score was chosen for inclusion.

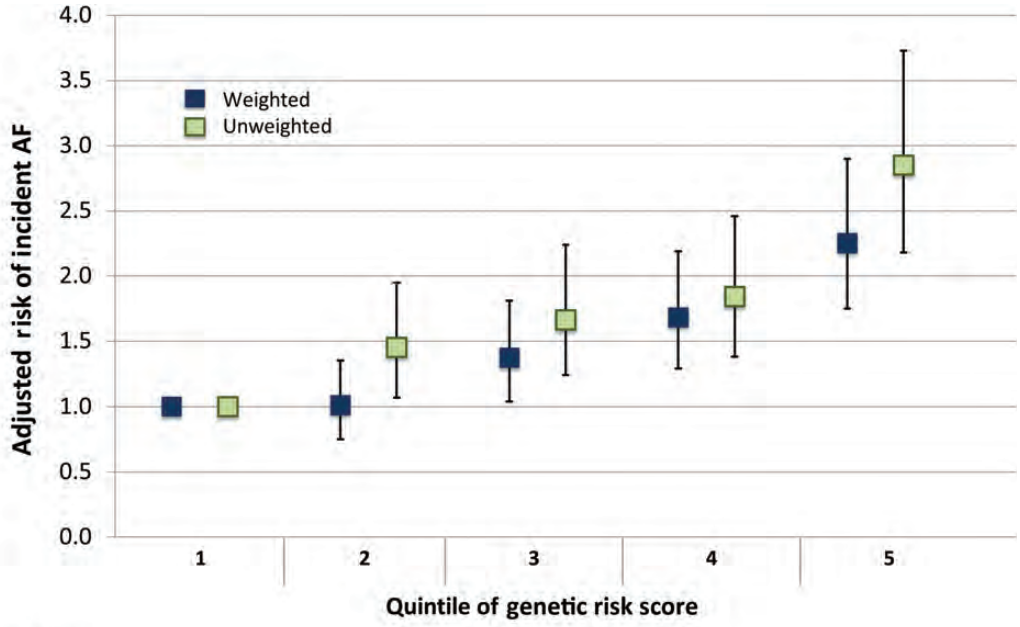
Clinical reclassification of atrial fibrillation with and without genetic information

When tested in the validation set, the addition of the weighted GRS to a model including age alone improved the C-index [0.704 (0.667–0.739), $P = 0.0006$], the NRI [0.107 (0.0286–0.1830), $P = 0.006$], the continuous NRI [0.459 (0.261–0.643), $P < 0.0001$], and the IDI [0.00474 (0.00316–0.00672), $P < 0.0001$] (*Table 4*). The addition of the weighted GRS to the WHS AF risk prediction algorithm improved AF risk prediction as measured by the C-index [0.741 (0.709–0.774), $P = 0.001$], the continuous NRI [0.490 [0.301–0.670), $P < 0.0001$], and the IDI [0.00526 (0.00625–0.00759), $P < 0.0001$]. However, reclassification into our pre-specified clinical risk categories did not improve after the addition of the weighted

Age–only model 10–year risk categories	WHS model 10–year risk categories <1%	1 to <5%	5+ %	Total	No. (%) Reclassified
<1 %					
Number of participants	2426	418	1	2845	419 (14.7)
Per cent of participants	35.3	6.1	0.01		
Kaplan–Meier event rate	0.66	1.72	0		
1 to <5%					
Number of participants	790	2745	222	3757	1012 (26.9)
Per cent of participants	11.5	39.9	3.2		
Kaplan–Meier event rate	0.39	1.89	7.95		
5+%					
Number of participants	0	115	162	277	115 (41.5)
Per cent of participants		1.7	2.4		
Kaplan–Meier event rate		2.73	7.72		
Total	3216	3278	385	6879	1546 (22.5)

Net reclassification improvement (95% CI): 0.211 (0.117–0.303); P<0.0001

Figure 1 Clinical reclassification of participants in the validation cohort for the age alone model when compared with the novel Women's Health Study atrial fibrillation risk prediction algorithm (WHS Model). In total, 1546 participants were reclassified, 1546 (100%) correctly. Reclassification χ^2 calibration statistics calculated from this table were 25.3 ($P = 0.0001$) for the age alone model and 4.51 ($P = 0.48$) for the novel Women's Health Study atrial fibrillation risk prediction algorithm.



Genetic risk score					
Weighted, range	<0.25	0.25 to <0.51	0.51 to <0.74	0.74 to <1.02	≥1.02
Unweighted, range	<9	9 to <10	10 to <11	11 to <12	≥12

Figure 2 Adjusted relative risk of incident atrial fibrillation for increasing quintiles of the weighted and unweighted genetic risk scores in the entire Women's Health Study cohort ($n = 20\,437$). Estimates of relative risk and 95% confidence intervals are adjusted for the covariates included in the Women's Health Study atrial fibrillation risk prediction algorithm [ln(age), weight, height, systolic blood pressure, alcohol use (≥2 drinks per day) and ever smoking status].

Table 4 Indices of model fit, calibration, discrimination, and reclassification in the validation cohort after the addition of genetic information to the age alone and WHS atrial fibrillation risk prediction algorithm

	Age alone	Age + AF weighted genetic risk score	*P*-value[a]	WHS alone	WHS + weighted AF genetic risk score	*P*-value[b]
Model fit (χ^2)[c]	55.3	62.7	–	87.9	104.1	
Model calibration [χ^2 (*P*-value)][d]	7.01 (0.54)	2.76 (0.95)	–	8.07 (0.43)	3.29 (0.91)	–
C-index (95% CI)	0.671 (0.636–0.710)	0.704 (0.667–0.739)	0.0006	0.718 (0.684–0.753)	0.741 (0.709–0.774)	0.001
NRI (95% CI)		0.107 (0.0286–0.183)	0.006		0.041 (−0.0444–0.123)	0.33
Continuous NRI (95% CI)		0.459 (0.261–0.643)	<0.0001		0.490 (0.301–0.670)	<0.0001
IDI (95% CI)		0.00474 (0.00316–0.00672)	<0.0001		0.00526 (0.00325–0.00759)	<0.0001

Coefficients used to test the models were calculated in the derivation cohort.
CI, confidence interval; IDI, integrated discrimination improvement; NRI, net reclassification improvement; WHS, Women's Health Study.
[a]*P*-value for comparison with the age alone risk prediction algorithm.
[b]*P*-value for comparison with the WHS risk prediction algorithm.
[c]All model fit likelihood ratio χ^2 statistics were highly significant ($P < 0.0001$).
[d]*P*-value of <0.01 required to reject the hypothesis that a model was well calibrated.

	WHS model plus the weighted AF genetic risk score 10–year risk categories				
WHS model 10–year risk categories	**<1%**	**1 to <5%**	**5+ %**	**Total**	**No. (%) Reclassified**
<1 %					
Number of participants	2907	309	0	3216	309 (9.6)
Per cent of participants	42.3	4.5			
Kaplan–Meier event rate	0.55	0.98			
1 to <5%					
Number of participants	470	2687	121	3278	591 (18.0)
Per cent of participants	6.8	39.1	1.8		
Kaplan–Meier event rate	0.43	1.93	6.84		
5+%					
Number of participants	0	111	274	385	111 (28.8)
Per cent of participants		1.6	4.0		
Kaplan–Meier event rate		6.63	8.30		
Total	3377	3107	395	6879	1011 (14.7)
Net reclassification improvement (95% CI): 0.041 (–0.0444–0.123); *P*=0.33					

Figure 3 Clinical reclassification of participants in the validation cohort for the Women's Health Study model plus the atrial fibrillation weighted genetic risk score, when compared with the Women's Health Study model without genetic information. In total, 1011 participants were reclassified, 591 (58.5%) correctly. Reclassification χ^2 calibration statistics calculated from this table were 6.12 ($P = 0.30$) for the Women's Health Study model and 3.96 ($P = 0.56$) for the Women's Health Study model plus the atrial fibrillation genetic risk score.

genetic score to the WHS AF risk prediction algorithm [NRI: 0.041 (−0.0444–0.123), $P = 0.33$]. Nevertheless, many (591, 58.5%) of the 1011 reclassified participants were reclassified correctly on the basis of the genetic information (*Figure 3*). In a secondary analysis, we observed similar results for the unweighted GRS when it was added to the WHS AF risk prediction algorithm (Supplementary material online, *Table S4* and *Figure S1*). Finally, in a sensitivity analysis conducted in the entire WGHS, we observed similar changes in the indices of reclassification after the addition of the GRS to the WHS AF risk prediction algorithm (Supplementary material online, *Table S5* and *Figures S2 and S3*).

Discussion

In this prospective cohort of 20 822 women without CVD at baseline, we derived and validated a novel WHS AF risk prediction algorithm, which despite being relatively simple, demonstrated good discrimination, calibration, and improved reclassification into 10-year risk categories when compared with age alone. We then tested whether our ability to predict incident AF was improved by the addition of a weighted or unweighted GRS to the risk prediction algorithm. The addition of either GRS improved the c-index and other continuous measures of risk discrimination, but did not appreciably improve the ability to classify participants into pre-specified 10-year risk categories.

Our AF risk prediction model derived among women without pre-existing CVD shares many AF risk predictors with those derived in the FHS and ARIC populations, which included men and women with and without established CVD.[6,7] The exceptions included physical exam findings and electrocardiographic variables, which were unavailable in this cohort. Despite the absence of this information, our model performed well and was able to reclassify 22.5% of women in a separate validation cohort. The six variables selected for inclusion in the AF risk prediction model—age, weight, height, systolic blood pressure, alcohol use, and past or current smoking—are readily available in nearly every primary prevention population. In addition, several of these variables are modifiable through lifestyle interventions. Therefore, patients can be counselled regarding lifestyle changes that might lower their 10-year risk of AF. Potential future clinical applications of this simple AF risk prediction algorithm could include identification of populations where targeted screening for asymptomatic AF might be cost-effective and/or where interventions designed to lower AF risk might be tested in randomized trials. Given the expanding indications for anticoagulation in lower risk populations[21,22] and advances in rhythm monitoring devices,[23] targeted screening for asymptomatic AF may have clinical utility in the near future.

When compared with the traditional AF risk factors described above, none of the 14 blood biomarkers we considered met our pre-specified criteria for inclusion in the WHS AF risk prediction algorithm, even though several, such as CRP and haemoglobin A1c, have previously been associated with AF in this or other cohorts.[9,10] While our inclusion of these biomarkers in the model derivation process is a strength of our study, B-type natriuretic peptide levels were not available for analysis. B-type natriuretic peptide levels have been strongly associated with incident AF[24,25] and improved the measures of discrimination when added to the FHS AF risk algorithm.[8] Whether they would offer similar improvements in risk prediction in our relatively healthy cohort of women is unclear and requires further study.

Data are sparse regarding the contribution of genetic data to AF risk prediction. Recently, investigators from the Malmo Diet and Cancer Study did not find an improvement in AF risk prediction, as measured by the C-statistic, when two genetic variants at two loci strongly associated with AF (4q25 and 16q22) were added to traditional risk factors.[26] In contrast, we found that a risk score comprised of 12 variants at nine AF loci improved several measures of AF risk prediction including the c-index, the continuous NRI, and the IDI in our population of women without established CVD. These data suggest that genetic information has the potential to improve the identification of individuals at higher risk for AF among healthy populations and raise the possibility that the inclusion of more genetic risk markers may improve our ability to predict AF in the future. Although the present GRS did not improve our ability to classify women into discrete 10-year AF risk categories, the continued search for additional genetic variants associated with AF may improve discriminatory ability in the future. Also, since there is currently no consensus regarding clinically meaningful AF risk categories, the continuous NRI and IDI may be more appropriate measures of model performance since they are not based upon arbitrary risk categories.[18,27,28] Regardless, the data presented here are not yet strong enough to justify widespread genetic screening to assess AF risk.

The strengths of our study include the size of the study population, the duration of follow-up, the number of prospectively ascertained and physician-validated AF cases, and the breadth of risk factors and biomarkers considered for inclusion in the model. In addition, we were able to validate both the AF risk prediction model and the contribution of genetic information to risk prediction, in a reserved validation cohort of women. To our knowledge, this has not been done previously with prior AF risk prediction scores.

Our study also has important limitations which merit consideration. The generalizability of our findings may be limited to women with a low prevalence of CVD and HF and to those of European ancestry. As such, the WHS AF risk score may not perform as well in other populations. This is a limitation common to risk scores and was found to be the case when the FHS AF risk score was applied to external populations.[29] Future studies are needed to validate our model in other populations and to determine if our strategy of using the BIC to select a small number of covariates for the model translates to good performance outside of the WHS.

Second, we did not collect baseline ECGs, and therefore, we were unable to evaluate whether information on PR interval, left atrial enlargement, and left ventricular hypertrophy, which have been included in other risk prediction algorithms[6,7] would add to AF risk prediction among women without CVD. Thus, we were unable to compare our model performance to that of the FHS and ARIC scores. We also did not perform screening ECGs during follow-up and some asymptomatic cases of AF may have gone undetected. Third, we did not collect information on the family history of AF at baseline and thus were unable to compare the predictive value of this information to that provided by the GRS.[17] Fourth, as mentioned above, although we were able to test numerous blood biomarkers for inclusion in our model, we were not able to test all blood biomarkers that have been associated with AF in our study population.

In conclusion, in this large-scale, prospective cohort of initially healthy women of European ancestry, we derived and validated a novel, simple AF risk prediction algorithm utilizing six easily measured AF risk factors (age, weight, height, systolic blood pressure, alcohol use, and smoking). Beyond this information, a GRS based on recently published risk alleles showed potential for improving the ability to identify individuals at higher risk for AF; however, we did not find definitive evidence that the currently identified AF risk alleles can be utilized as a clinically meaningful risk

stratification tool at present. Discovery of additional genetic variants and/or application to targeted populations may improve the clinical performance of GRSs. At the same time, research directed at developing effective AF screening and prevention strategies will increase the clinical impact of AF risk prediction scores.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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CARDIOVASCULAR FLASHLIGHT

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Granulomatous mass adherent to a patent foramen ovale occluder

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A 70-year-old woman with a history of repeated cerebrovascular events and patent foramen ovale (PFO) closure with PREMERE occluder (St Jude Medical, St Paul, MN, USA) had a recurrence of transient left hemi paresis. Transoesophageal echocardiography (TOE) revealed an intracardiac mass on the left side of the PFO closure system (8 × 8 mm) (*Panel A*). Despite oral anticoagulation, TOE performed 2 months later found persistent mass (*Panel B*). Surgical removal of the PFO occluder with the linked mass (*Panel C*, arrow) and the closure of the atrial communication were, therefore, performed. Histology concluded to a granulomatous formation. Exogenous structures (*Panel D*) that may come from the occluder were found in the tissue and participated to an inflammatory reaction associated with fibrosis. To our knowledge, this is the first report of a granulomatous mass which needed removal of a PFO occluder. Beyond its debated indication, this highlights that the PFO closure may have rare but still unknown and not negligible complications.